Mycobiota of sunflower seeds and samples collected from vegetable oil refinery located in Tamilnadu, India

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Sunflower seeds and its products are widely consumed as fodder and vegetable oil in India. The mycobiota is known to produce hazardous effects to the consumer by producing toxins. Hence the mycobiota starting from seed stage to kernel, Oiled Cake (OC), De-Oiled Cake (DOC), Solvent Extracted Oil (SEO), Expeller Oil (EO) and Refined Oil (RO) were studied using Czapek-Dox agar. Altogether 24 non-xerophilic species belonging to 12 genera were isolated. The fungus *Aspergillus flavus* was found to be predominant in seeds with 22.3 % contribution to the total. In the kernel, *Mucor racemosus* was dominant and contributed 31.6 %. In OC and DOC, *Rhizopus stolonifer* was dominant with 31.1 % and 45.9 % respectively. In EO and SEO, *Aspergillus flavus* and *A. japonicus* were dominant with 21 % and 32.4 % respectively. It was found that there was no fungal growth in RO. The study was conducted between the periods 2000–2001.

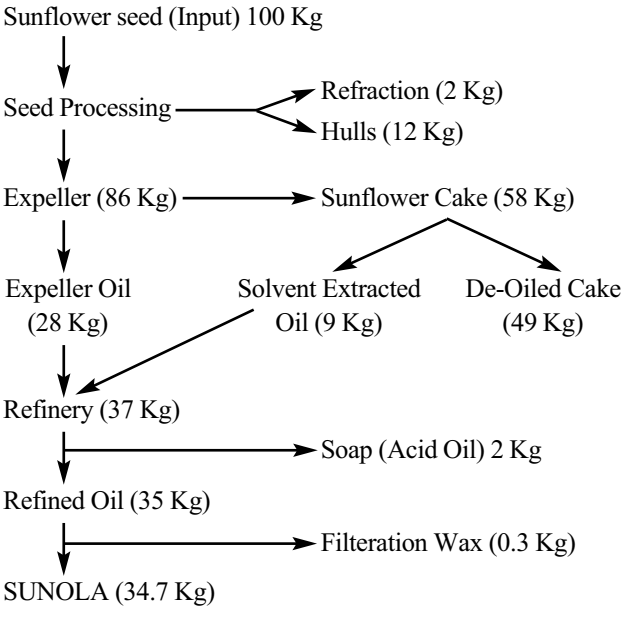
Keywords: Aflatoxin, *Aspergillus flavus*, expeller oil, moisture, refined oil, solvent extracted oil, sunflower seeds, sunflower kernels, sunflower oil cake, sunflower de-oiled cake

Several investigations have been carried out to enumerate the mycobiota of various kinds of foodstuff (ABDEL-HAFEZ 1984; GIRISH & GOYAL 1986; ASEVEDO et al. 1994; SAHIN & KALYONUROGLU 1994; FREIRE, KOZAKIEWICZ & PATERSON 2000; MAHMOUD 2000; BUENO, SILVA & OLIVER 2001; MARTIN 2001). Sunflower oil is one of the most important edible oil used by Indians because of the low cholesterol content. There are many reports listing several fungi associated with sunflower seeds (RAUT 1955; NAGARAJAN, BHAT & TULPULE 1974; SURYANARAYANAN & SURYANARAYANAN 1990). Although sunflower seeds and the corresponding products may be highly contaminated with aflatoxin (RANJAN 1986; LUCA et al. 1989; CHULZE et al. 1991; BABILA & AKCADAG 1991; VIJAYALAKSHMI & RAO 1993; JAND & SINGH 1995), no detailed information on the potentially toxigenic mycobiota other than aflatoxigenic fungi is available. On these lines, it is planned to investigate the fungal population in the seed used to extract oil in the refinery to obtain a comparative nature of the mycobiota in the seed population. Any mycotoxin producing fungus isolated will be given special emphasis for further study.

Material and methods

Samples were collected once in two months from the site mentioned. Fresh samples of sunflower seeds, kernels, Oil Cake (OC), De-Oiled Cake (DOC), Expeller Oil (EO), Solvent Extracted Oil (SEO) and Refined Oil (RO) were collected during milling from Tamil Nadu Agro Industries Corporation Ltd., situated in Pochamppalli, Tamil Nadu, India.

Refined oil recovery chart of the Sunflower seed



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Tab. 1: Fungi isolated from seeds and kernels

Name of the fungus	Seeds		Kernels	
	Average CFU/g	% Contribution	Average CFU/g	% Contribution
Zygomycotina				
Mucor mucedo	0.36	1.99	–	–
Mucor racemosus	2.11	11.50	7.10	31.60
Rhizopus stolonifer	3.75	20.30	3.40	15.20
Ascomycotina				
Emericella nidulans	0.06	0.36	–	–
Deuteromycotina				
Aspergillus flavus	4.10	22.30	3.50	15.70
Aspergillus fumigatus	1.90	1.63	0.25	1.10
Aspergillus glaucus	–	–	0.25	0.11
Aspergillus japonicus	0.90	5.07	0.15	0.66
Aspergillus nidulans	0.10	0.63	–	–
Aspergillus niger	3.50	19.00	4.50	19.90
Aspergillus ochraceus	0.18	0.99	0.47	2.10
Aspergillus tamarii	0.01	0.09	–	–
Curvularia lunata	0.01	0.09	–	–
Penicillium citrinum	0.21	1.17	0.27	1.20
Penicillium chrysogenum	0.01	0.09	0.03	0.11
Penicillium funiculosum	–	–	0.03	0.11
Penicillium islandicum	0.88	4.80	–	–
Trichoderma sp.	0.28	1.54	–	–
Colletotrichum gleosporioidus	0.01	0.09	–	–
Phoma sp.	0.15	0.81	0.05	0.22
Non-sporulating Colonies	0.26	1.45	0.12	0.55

Seeds were harvested from 3 months old plant during April-May, and December-January belonging to the variety of Gargil, Mico-17 and Maharastra were purchased from the growers in bulk and they were mixed together and crushed for oil milling. Seeds were stored at the temperature of 30–40 ºC and DOC and OC were stored at 38 ºC. Moisture level of seeds is 6 % or below 6 %. For OC the moisture content is ± 6 % and for DOC, it is 9 %. Oil samples were maintained at the moisture level of 0.1 %. Samples were collected in sterile polyethylene bags and in conical flasks and stored at 30 ºC in the laboratory up to 60 days.

Mycobiota Analysis

From each collection (total 6 collections), 7 seeds per plate usually in triplicates (total 126 seeds) and 7 kernels per plate usually in triplicates (total 126 kernels) were surface sterilized for 2 min. in 0.1 % mercuric chloride solution, for to enumerate the storage fungi and washed thoroughly in sterile distilled water for 2 min. and plated on to Czapek-Dox Agar (CDA). For DOC and OC (total 180 gms of each), 10 gms of each sample were shaken thoroughly in 100 ml (101) of sterile distilled water for 15 min. From this sample, 1 ml was taken and plated onto CDA medium. 1 ml of SEO, EO and RO (total 18 ml of each) were plated directly on to the medium. The plates were incubated at 30 ºC up to one week.

The individual species of fungi were counted separately, and their numbers were expressed as Colony Forming Units per gram (CFU/g). The Aspergilli and Penicillia were identified following (PITT 1979; ONIONS, ALLSOPP & EGGINS 1981). Nomenclature of Aspergilli was made after RAPER & FENNELL (1965). The abundance, density, % frequency, % occurrence and % contribution were calculated for each species (VITTAL 1973). The strains were deposited in the Madras University Botany Laboratory (MUBL), University of Madras, Chennai - 600 025.

Results

Mycobiota of the Seeds

20 species classified under 9 genera were isolated from the seeds, which included 3 Zygomycetes, 1 Ascomycete, 14 Hyphomycetes and the remaining Coelomycetes. Of the 20 species, only 7 species viz., *Aspergillus flavus, A. niger, A. fumigatus, A. japonicus, A. ochraceus, A. tamarii, Penicillium citrinum* and *Rhizopus stolonifer* were recorded more fre-

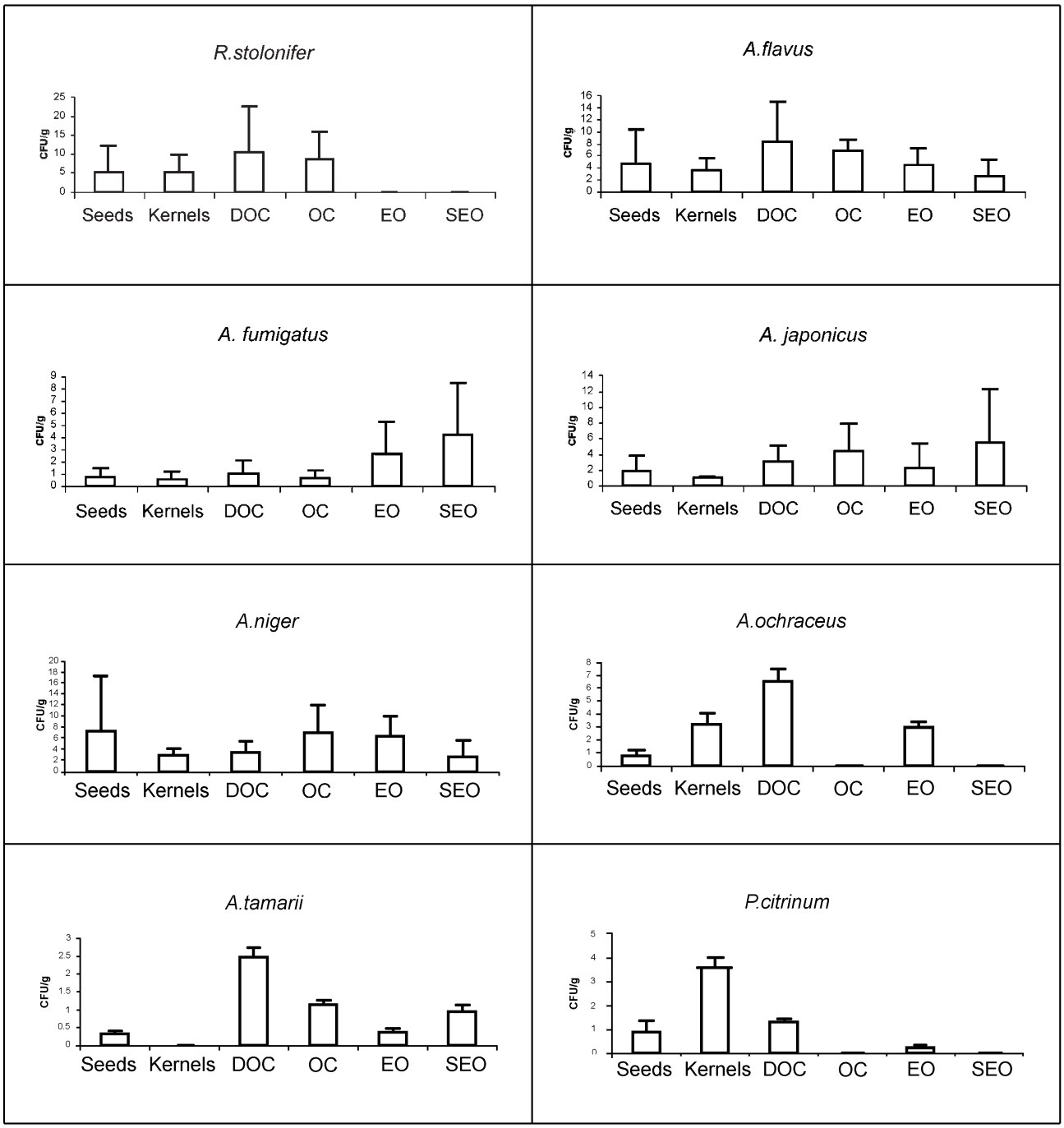

Fig. 1: Distribution of some selected fungi in different samples

quently (Fig. 1). The species are listed in Tab. 1 along with their colony forming units (CFU) per gram of sample with their percentage contribution to the total seed mycobiota.

Aspergillus flavus showed maximum contribution (22.3 %) and appears to be the most predominant species in the seed. *Rhizopus stolonifer* occupied the second position with 20.3 %. *A.niger* occupied the third position with 19 %. The percent contribution of other fungi were 32.2 %. As a genus, all the species put together, *Aspergillus* accounted for 49.71 % and *Penicillium* for 6.06 % and the rest by other genera (Fig. 2).

Mycobiota of the Kernels

Altogether 12 species belonging to 5 genera were isolated from the kernels (Tab. 1). These included 2 Zygomycetes, 9 Hyphomycetes and the remaining 1 was a Coelomycete. Of these, *Aspergillus flavus, A. niger, Penicillium citrinum* and *Rhizopus stolonifer* were recorded more frequently (Fig. 1).

Mucor racemosus was abundant in kernel and contributed the maximum percentage (31.6 %) to the total. Although *A. niger* occupied the second position its percentage contribution was much lower (19.9 %) when compared to *M. racemosus.*

Tab. 2: Fungi isolated from oil cake (oc) and de-oiled cake (doc)

Name of the fungus	OC		DOC	
	Average CFU/g	% Contribution	Average CFU/g	% Contribution
Zygomycotina				
Absidia corymbifera	0.60	1.87	–	–
Mucor mucedo	–	–	2.84	7.15
Mucor racemosus	–	–	0.03	0.07
Rhizopus stolonifer	10.00	31.10	18.20	45.90
Syncephalastrum racemosum	–	–	0.03	0.07
Ascomycotina				
Emericella nidulans	0.20	0.62	–	–
Deuteromycotina				
Aspergillus flavus	6.70	20.90	7.90	20.00
Aspergillus fumigatus	0.60	2.00	0.50	1.41
Aspergillus japonicus	4.40	13.70	1.40	3.61
Aspergillus niger	7.40	23.20	5.50	13.80
Aspergillus ochraceus	–	–	0.12	0.31
Aspergillus tamarii	0.20	0.83	0.03	0.07
Aspergillus terreus	0.20	0.62	0.68	1.70
Monilia sitophila	1.00	3.10	0.18	0.47
Penicillium citrinum	–	–	0.12	0.31
Penicillium islandicum	–	–	0.03	0.07
Non-sporulating Colonies	0.50	1.66	1.98	4.80

A. flavus occupied the second position and contributed 15.7 %. The percentage contribution of remaining genera was very low. As a single genus *Aspergillus* accounted for 39.57 % of the total kernel mycobiota (Fig. 2).

Mycobiota of Oil Cake (OC)

10 species classified under 5 genera were isolated from the OC, which included 2 species of Zygomycetes, 1 belonged to Ascomycete and the remaining 7 belonged to Deuteromycetes (Tab. 2).

Rhizopus stolonifer showed maximum contribution (31.10 %) and appears to be the most predominant species. *A. niger* occupied the second position with 23.2 %. The other species with a notable contribution was *A. flavus*, which occupied the third position (20.90 %). As a genus *Aspergillus* with all put together accounted for 60.42 % to the total (Fig. 2).

Mycobiota of De-Oiled Cake (DOC)

14 species classified under 6 genera were isolated from the de-oiled cake, which included 4 Zygomycetes and the remaining Hyphomycetes (Tab. 2). Of the 14 species, only 3 species, viz., *A. flavus, A. niger* and *R. stolonifer* were recorded more frequently (Fig. 1).

R. stolonifer showed maximum contribution (45.9 %) and appears to be the most predominant species. It is followed by *A. flavus* with 20 %. *A. niger* occupied the third position (13.8 %). The percent contribution of other fungi was 20.32 %. As a genus *Aspergillus* accounted for 40.9 % (Fig. 2).

Mycobiota of Expeller Oil (EO)

14 species classified under 5 genera were isolated from the EO, which included 2 Zygomycetes and rest were Hyphomycetes (Tab. 3). Among this, *A. flavus, A. japonicus, A. niger, A. fumigatus* and *M. racemosus* occurred very frequently (Fig. 1).

A. flavus was abundant and contributed the maximum percentage (21 %) to the total. *A. japonicus* occupied the second position and contributed 15.6 %. The third position was occupied by *A. fumigatus* and contributed 13.1 %. As a genus, *Aspergillus* contributed 70.08 % to the total EO mycobiota (Fig. 2).

Mycobiota of Solvent Extracted Oil (SEO)

A total of 6 species belonging to 2 genera were isolated from SEO (Tab. 3). There was no representative from Zygomycetes, whereas in Deuteromycetes, the genus *Aspergillus* had 5 species and *Trichoderma* had 1 species. Of the 6 species, *A. japonicus* and *A. fumigatus* were recorded frequently (Fig. 1).

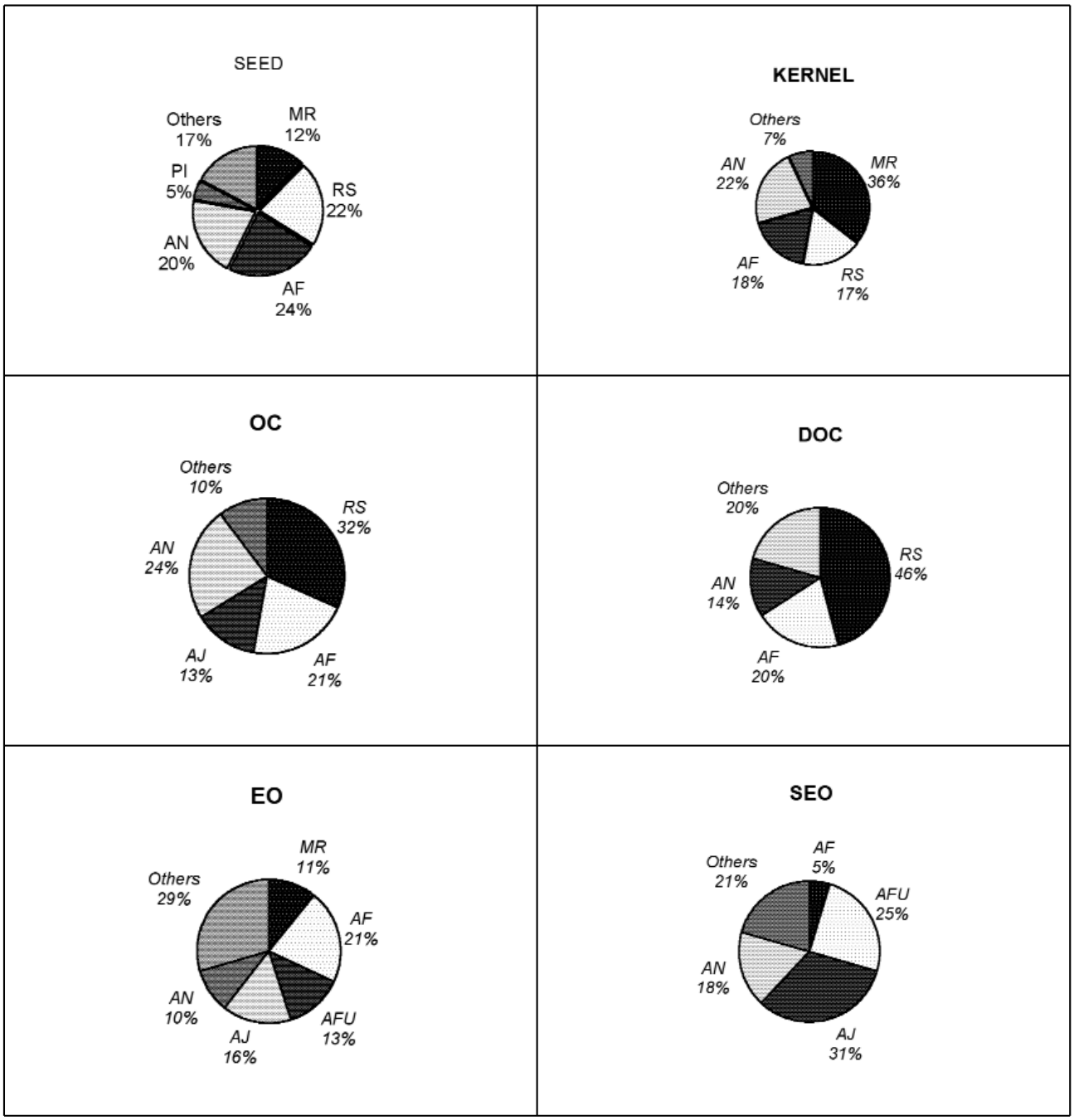

Fig. 2: Species of fungi frequently isolated from different samples and their percentage contribution

MR = *Mucor racemosus* AFU = *Aspergillus fumigatus* PI = *Penicillium islandicum*
RS = *Rhizopus stolonifer* AJ = *Aspergillus japonicus*
AF = *Aspergillus flavus* AN = *Aspergillus niger*

Aspergillus japonicus showed the maximum contribution (32.40 %) to the total. *A. fumigatus* occupied the second position and contributed 24.60 %. The percentage contributions of other fungi were 42.44 %. As a genus, *Aspergillus* contributed 82.66 % to the total mycobiota (Fig. 2).

Mycobiota of Refined Oil (RO)

There was no growth of fungi in refined oil (Tab. 3).

Discussion

Sunflower is native to North America where it was used in dyes, food preparation and medicines. It then spread throughout the world and developed as an oilseed crop in Russia during the late 1800s. The oil has found widespread acceptance as a high quality, edible oil throughout much of the world. Major producing countries or areas are the Former Soviet

Tab. 3: Fungi isolated from expeller oil, solvent extracted oil & refined oil

Name of the fungus	Expeller Oil		Solvent Extracted Oil		Refined Oil	
	Average CFU/g	% Contribution	Average CFU/g	% Contribution	Average CFU/g	% Contribution
Zygomycotina						
Mucor mucedo	0.31	1.18	–	–	–	–
Mucor racemosus	2.95	10.96	–	–	–	–
Deuteromycotina						
Aspergillus flavus	5.70	21.00	0.75	4.68	–	–
Aspergillus fumigatus	3.50	13.10	3.90	24.60	–	–
Aspergillus japonicus	4.20	15.60	5.10	32.40	–	–
Aspergillus niger	2.70	9.94	2.80	17.50	–	–
Aspergillus ochraceus	1.22	4.55	–	–	–	–
Aspergillus tamarii	0.13	0.50	0.18	0.78	–	–
Aspergillus terreus	1.40	5.39	0.40	2.70	–	–
Monilia sitophila	1.63	6.07	–	–	–	–
Penicillium citrinum	0.04	0.16	–	–	–	–
Penicillium funiculosum	0.40	1.51	–	–	–	–
Penicillium oxalicum	0.04	0.16	–	–	–	–
Trichoderma sp.	0.04	0.16	0.12	0.78	–	–
Non-sporulating Colonies	2.50	9.44	2.50	16.00	–	–

Union (FSU), Argentina, Eastern Europe, USA, China, France, and Spain.

Currently, there are basically two types of sunflower seed produced, oil and confectionary. World sunflower seed production has increased from an average of 23.5 million tones (Mt) in the mid 1990s to 26.9 Mt estimated by the USDA for 1999 – 2000 (de RODRIGUEZ et al. 2002). In 2002 - 2003, the world production of sunflower seed was 22.9 Mt. In 2004, it is estimated to about 25,960,000 tons. Sunflower though a recent introduction as an oilseed crop in India, has gained importance and popularity due to its suitability to various regions of the country. The total land under sunflower cultivation is 2 million hectares and the total production was 1.17 million tones in the year 1998-99. Oil production of 3,38,000 tonnes was recorded in 1997-98, which reduced from 4,50,000 tonnes in 1996-97 (ANITHA 2001). In Tamil Nadu, 24,299 hectares of land is under cultivation, which produces 13,660 tonnes of seeds. The major sunflower producing states in India are Maharastra, Andhra Pradesh and Karnataka. No reports are available from the producing countries on the incidence of aflatoxin in domestic sunflower seed or cake. However, in Hungary, 9.6 % of 73 samples analysed in 1975 showed contamination with aflatoxin (ANON. 1989). In Germany, analysis of 4 samples of sunflower cake showed one sample with 17 µg/kg aflatoxin (ANON. 1989). In Italy two samples of sunflower seeds analysed were found to contain 50–90 µg/kg of aflatoxin B (ANON. 1989).

MILLER, PRETORIUS & TRINDER (1985) proposed a simple method of aflatoxin determination in vegetable oils. The method was successfully applied to both crude and degummed oils. As quantitated by thin layer chromatography and liquid chromatography, the oils analyzed contained aflatoxin B1 at levels of 5–200 µg/kg.

KAMIMURA et al. (1986) reported that during the survey of mycotoxin contamination in edible oil and fate of mycotoxin during oil refining process, the mycotoxin are eliminated during neutralization process including alkaline refining and washing and decolourization process.

ETCHEVERRY et al. (1989) reported that water activity increased during storage of the sunflower seeds and the germinability decreased. There was an increase in the free fatty acid content and the aflatoxin B increased from 205 to 520 µg/kg.

BABILA & AKCADAG (1991) reported the presence of aflatoxin B1 (16 ppb) and Ochratoxin A (800 ppb) in the sunflower seed feeds used in a poultry farm, which caused mycotoxicosis in the birds.

The preparations made from deoiled meal have been used as protein-rich supplements to pre-school children (NAGARAJAN, BHAT & TULPULE 1974). But sunflower seeds were shown to support aflatoxin production (B1). Toxin production was reported to be more in broken seeds than in whole seeds. The reason for this may be that the whole seeds may not harbour enough fungi whereas the broken seeds may contain a large number of fungal spores.

Mycotoxins are a group of highly toxic secondary metabolite of the fungi. E.g. Aflatoxin B1, B2, G1 and G2, Ochratoxin, Citrinin, T–2 toxin, Patulin, Fumonisin, Deoxynivalenol,

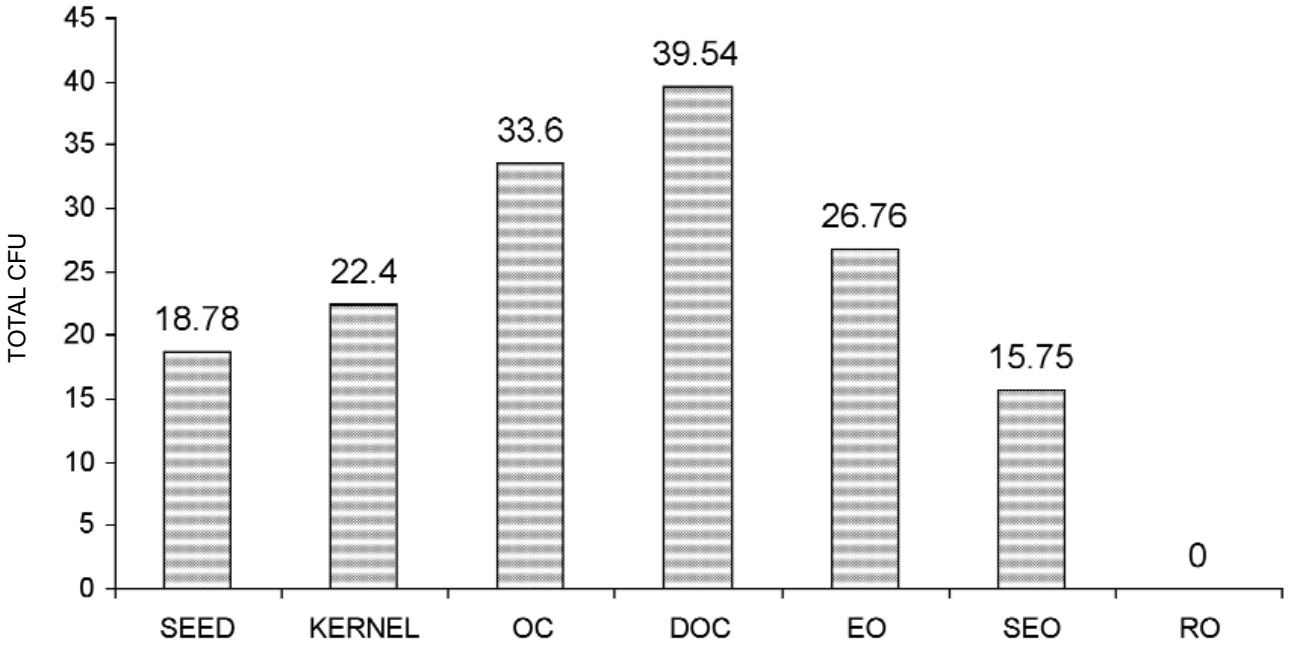

Fig. 3: Total CFU of different samples

Luteoskyrin and so on. It has been well documented that mould contaminated food are often responsible for animal mycotoxicoses as a result of ingestion of mycotoxin (KROGH, HALD & PEDERSEN 1973; CIEGLER, HAYES & VESONDER 1980). It is impossible to remove all traces of fungal spores from the oiled and deoiled cake samples but precaution should be taken to improve quality and decrease the probability of mycotoxin contamination.

The present study with the sunflower seeds samples suggests that except the refined oil, none of the other samples collected was free from fungal infection. Although their incidence varied with the different samples (Fig. 3). This might be caused by the differences in their moisture contents. Seeds and kernels showed medium levels of fungal contamination due to their moisture content 6 % (or) below 6 % and it was slowly increased with the increase in moisture content for oil cake ± 6 %, for de-oiled cake contamination of fungi increased suddenly corresponding to the increase of moisture content (9 %) and it was observed decreasing for oil samples with the decreasing moisture content. High levels of aflatoxins were found in sunflower seeds in Tunisia. This high levels were due to lack of refining and low levels were reported for refined food oils (ANON. 1978).

Altogether 7 different species of *Aspergillus* are reported from various samples. *Aspergillus flavus* is reported from seed, kernel, oiled and deoiled cake, and expeller and solvent extracted oil samples. Species of *Aspergillus* are of great importance as they produce the aflatoxins, the most important of all mycotoxin (BUSBY & WOGAN 1981). *Aspergillus flavus* is the main source of aflatoxins, the most important mycotoxin in the world's food supplies. Aflatoxins are toxic to animals including man. Because of their high toxicity, low limits for aflatoxins in foods and feeds have been set by most countries (EGMOND 1989; JELINEK, POHLAND & WOOD 1989; GOURAMA & BULLERMAN 1995). The universal occurrence of *A. flavus* in commodities such as peanuts, maize, cottonseeds, pistachio nuts, and fig fruits has already been reported (BILGRAMI & SINGH 1984). The ability of *A. flavus* to grow as a nondestructive pathogen in the tissues of a variety of plants is already established (PITT & HOCKING 1985, 1996). Since it is reported that individual spores of *A. flavus* contain high concentrations of aflatoxin up to 1000 µg/kg. The contribution of *A. flavus* to the mycobiota of the present study showed 22.30 % in seed, 15.70 % in kernel, 20.90 % in oiled cake, 20 % in deoiled cake, 21 % in expeller oil and 4.68 % in the solvent extracted oil. The de-oiled cake contains 20 % contribution of *A. flavus*, which is not suited for using as animal or poultry feed.

Among the other species of *Aspergillus,* only *A. niger* showed the highest % of contribution in all the samples (Tables 1, 2, 3) whereas *A. fumigatus, A. glaucus, A. japonicus, A. nidulans* (asexual stage), *A. ochraceus, A. tamarii* showed lower % of contribution in the samples. *A. niger* is usually regarded as a safe species though two of its isolates were recently reported to produce ochratoxin A (ABARCA et al. 1994). *A. niger* is the most common fungus isolated from peanuts, walnuts, coconuts and copra (MORNTTE, PALOMAR & LIM 1986). The presence of *A. fumigatus, A. glaucus, A. japonicus, A. nidulans, A. ochraceus* and *A. tamarii* in the various samples is also correlated with the moisture content and other chances of contamination from the storage systems.

Curvularia lunata, though it is a field fungi it is reported only from the seed samples, the % of contribution being 0.09 %.

No reliable report of mycotoxin production by this fungus is known. It has already been reported to occur on rice, wheat, litchi fruit, walnuts, peanuts and spices (PITT & HOCKING 1997).

Five different species of *Penicillium* i.e. (*P. citrinum, P. chrysogenum*, *P. funiculosum, P. islandicum, P. oxalicum*) are reported from various samples. *Penicillium citrinum* is reported from seeds, kernel, deoiled cake and expeller oil. This is a mesophilic species and is known to produce citrinin, a mycotoxin of moderate toxicity (FRIIS, HASSELAGER & KROGH 1969). This toxin is a renal toxin to domestic animals. Citrinin causes watery diahorhoea, increased food consumption and reduced weight gain due to kidney degeneration in chickens (MEHDI, CARITON & TUITE 1981). Since the present study reports *P. citrinum* in the samples, it is adviced that the deoiled cakes which contain the fungus (0.31 % contribution) are not a suitable chicken feed. *Penicillium chrysogenum* is reported from seed and kernel samples. This is a mesophilic species and is reported from cereals, flour, ham, fish and spices (PITT & HOCKING 1997) and is not known as a pathogen. *Penicillium funiculosum* is reported from the kernel and expeller oil samples. This is an acid tolerant species, and is known to produce the mycotoxin patulin (VISMER et al. 1996). The species has been isolated from fruits, nuts and cereals (PITT & HOCKING 1997). *Penicillium islandicum* is reported from seed and deoiled cake samples. The fungus is known to produce at least four mycotoxins, unique to the species. Cyclochlorotine and islanditoxin produced by this species are very toxic. Luteoskyrin and erythroskyrin are known to be liver and kidney toxin (PITT & HOCKING 1997). It is a xerophile and an active agent in spoilage of cereals. It is reported from rice (MANABE & TSURUTA 1978), peanuts (JOFFE 1969) and soybean (MISLIVEC & BRUCE 1977). The toxin production in the spoilage of food is not yet confirmed.

Penicillium oxalicum is reported only in expeller oil. It has been reported from maize, rice, cowpea, peanuts, sorghum, black pepper, coriander etc. It produces secalonic acid D which is supplied to be toxic to animals (PITT & HOCKING 1997; CIEGLER, HAYES & VESONDER 1980).

Trichoderma sp. is reported from seeds and expeller oil. Many *Trichoderma* produce powerful chitinaces, cellulases and the toxin trichodermin and also exhibits mycoparasitism (PITT & HOCKING 1997). *Colletotrichum gloeosporioides* is reported from the seeds, which is usually known to produce anthracnose of fruits. Mycotoxin are not known to be produced by *C. gloeosporioides* (PITT & HOCKING 1997). Similarly, *Phoma* sp. is reported only from seeds and kernel samples, *Phoma* species are most likely to cause spoilage in weather damaged cereals (PITT & HOCKING 1997). *Monilia sitophila* is reported from oiled and deoiled cake samples and also from expeller oil. It is already reported in peanuts, sorghum and maize, soybean and cashews (PITT & HOCKING 1997). No toxin is reported to be produced by this fungus.

Among the Zygomycotina, 2 *Mucor* spp. namely, *M. mucedo* and *M. racemosus*, one species of *Rhizopus,* namely *R. stolonifer*, one species of *Absidia* , i.e. *A. corymbifera*, one species of *Syncephalastrum* i.e. *S. racemosum* are reported. *Mucor mucedo* has been reported from seed, deoiled cake and expeller oil samples respectively and is absent in kernel, oiled cake, solvent extracted oil and refined oil. But *M. racemosus* is reported in seed, kernel, deoiled cake and expeller oil samples. *Rhizopus stolonifer* was reported from seed, kernel oiled and deoiled cake samples. *Syncephalastrum racemosum* is reported only in deoiled cake sample. The incidence of various fungi belonging to the Zygomycotina on the seed, kernel, oiled and deoiled cake samples may be attributed to the diverse abiotic factors operating in the storage systems. It is probable that the moisture content in the bulk samples favoures the growth of these fungi (JAYARAMAN & KALYANASUNDARAM 1994). Though the above mentioned fungi were not reported to be aflatoxin producers, other mycotoxin producers like *A. flavus* and *A. niger* are encouraged to produce more of their toxin by the biological properties of co-invading fungal partner (CHOURASIA 1995). Non-sporulating colonies were found in seeds, kernels, oiled cake, deoiled cake, expeller oil and solvent extracted oil samples.

Conclusions

Moisture is the most important factor in determining if and how rapidly molds will grow in the samples. When compared to oil samples, seeds, kernels, Oil Cake and De-Oiled Cake contain high moisture. The high moisture content favours the growth of more fungi in seeds, kernels, Oil Cake and De-Oiled Cake than in oil and thereby increasing mycotoxin production. The final product i.e. the refined oil is free from any fungal contamination suggesting that the refining process under high temperatures (240 ºC for 6 hours) destroys the fungal spores present in the raw oil extracted from the seed and it was stored at the moisture level of 0.1 %. The oiled and de-oiled cakes are fed to animals which are found to have high level of mycotoxins produced by their fungal component. Therefore, it is suggested that severe scrutinization of the samples to be given to the animals should be done. Also, it is found that the refined oil is safe for consumption since there is no fungal growth from these samples and no possibility of mycotoxin production.

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